IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No: 14677-003

Applicant(s):	Klaus Giese *et al.*	Confirmation No.: 6369
Serial No.:	10/633,630	Examiner: Kimberly Chong
Filing Date:	August 5, 2003	Group Art Unit: 1635
Title:	INTERFERING RNA MOLECULES	

DECLARATION UNDER 37 C.F.R § 1.131

We, Klaus Giese, Anke Klippel-Giese and Jörg Kaufmann, do hereby declare as follows:

1. We understand that the claims in the captioned application have been rejected over U.S. patent application 2003/0190635, which lists February 20, 2002 as the earliest priority date, and U.S. patent application 2005/0142535, which lists February 1, 2002 as the earliest priority date.

2. We submit this declaration, based on our personal knowledge to demonstrate that the invention claimed in the captioned application was completed prior to February 2002 and therefore prior to the earliest prior art date of either U.S. patent application 2003/0190635, or 2005/0142535. All dates on the attached Exhibits have been masked out.

3. The delivery receipt attached as EXHIBIT 1 shows that two complementary oligoribonucleotide molecules, 94A2 and 94B1 were synthesized prior to February 1, 2002. 94A2 is the antisense strand and 94B1 is the sense strand. These molecules are described in the specification of the captioned application in Figure 15 where they are shown as PTENA V15 and PTENB V15.

4. In the delivery receipt, the lower case letters a, u, c, and g indicate the conventional unmodified ribonucleotides adenosine, uracil, cytosine, and guanosine, respectively. The number 5 indicates 2'O-methyl-ribo U, number 6 indicates 2'O-methyl-ribo A, number 7 indicates 2'O-

methyl-ribo C, and number 8 indicates 2'O-methyl-ribo G. Accordingly, the sequences of the two oligoribonucleotides are:

94A2: 5'-CuCcUuUuGuUuCuGcUaAcG-3' and

94B1: 5'-cGuUaGcAgAaAcAaAaGgAg-3'.

In these sequences a lower case letter indicates the unmodified nucleotide and the upper case indicates the 2'-O-methyl nucleotide. The nucleosides are linked by phosphodiester bonds.

5. Comparison of the sequences of 94A2 and 94B1 shows that they are both 21 nucleotides long, are complementary and have contiguous alternating 2'-O-methyl modified and single unmodified ribonucleotides, where a modified ribonucleotide on one strand is base paired with an unmodified ribonucleotide on the second strand and vice versa. 94A2 is complementary to a part of the PTEN gene. When 94A2 and 94B1 are combined, the resulting double stranded molecule is blunt ended.

6. Prior to February 1, 94A2 and 94B1 were combined to form a double stranded molecule designated 94A2/94B1 and demonstrated to inhibit expression of PTEN as shown in EXHIBIT 2. The results shown in EXHIBIT 2 were recorded prior to February 1, 2002.

7. EXHIBIT 2 shows the results of an experiment on the ability of RNAi molecules to inhibit PTEN expression in HeLaB cells. PTEN mRNA was measured using quantitative real time PCR analysis and was normalized to p110a mRNA as control. The bar graph in EXHIBIT 2 shows the results obtained with various double stranded RNA molecules. The 80AB molecule was an unmodified blunt siRNA molecule, and represented a positive control. The 79AB molecule was a completely 2'-O-methyl modified siRNA molecule and served as a negative control, . All molecules were tested at four different concentrations: 40 nM, 10 nM, 5 nM, 2,5 nM.

8. As can be seen from the data, molecule 94A2/94B1 shows activity that is very similar to the positive control 80AB, demonstrating that the molecule was effective at reducing PTEN mRNA expression in cells.

9. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Feb 10, 2008
Date

Klaus Giese

Feb 10, 2008
Date

Anke Klippel-Giese

Feb. 08/2008
Date

Jörg Kaufmann

EXHIBIT 1 of

EXHIBIT A

BioSpring

Lieferschein - delivery note

Oligonukleotidservice
Tel: 069 4109 2563
Fax: 069 4109 2564
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atugen AG / Haus 80

Robert-Rössle-Str.10

D-13125 Berlin

Ihre Bestnr. **Kundennummer** 10003
Lieferscheinnr. 3925 **Bestellung vom** [redacted]

1 94A1

5'-c5c 7u5 u5g 5u5 c5g 7u6 a7g-3'

IDO	16893	**OD**	12,9 **bei** 260 nm	**Länge**	21-mer
Scale	0,2 µmol	**Menge**	35361 pmol	**Molekular Gew.**	5348,20
Reinigung	Modifikation		35 nmol	**GC Gehalt**	29 %
DNA-Typ	DNA		189 µg	**Tm (GC)**	39 °C
				ε (mM)	364,810

Zusammensetzung A= 1,0 C = 3,0 G = 3,0 T = 4,0 **Modi** = 10,0

Mod. 5'	**Mod. 5** 2'OMe-rU	**Mod. 7** 2'OMe-rC
Mod. 3'	**Mod. 6** 2'OMe-rA	**Mod. 8**

2 94A2

5'-7u7 c5u 5u8 u5u 7u8 c5a 6c8-3'

IDO	16894	**OD**	33,4 **bei** 260 nm	**Länge**	21-mer
Scale	0,2 µmol	**Menge**	88514 pmol	**Molekular Gew.**	4799,86
Reinigung	Modifikation		89 nmol	**GC Gehalt**	14 %
DNA-Typ	DNA		425 µg	**Tm (GC)**	34 °C
				ε (mM)	377,340

Zusammensetzung A= 1,0 C = 3,0 G = 0,0 T = 6,0 **Modi** = 11,0

Mod. 5'	**Mod. 5** 2'OMe-rU	**Mod. 7** 2'OMe-rC
Mod. 3'	**Mod. 6** 2'OMe-rA	**Mod. 8** 2'OMe-rG

3 94B1

5'-c8u 5a8 c6g 6a6 c6a 6a8 g6g-3'

IDO	16910	**OD**	18,9 **bei** 260 nm	**Länge**	21-mer
Scale	0,2 µmol	**Menge**	56445 pmol	**Molekular Gew.**	6523,12
Reinigung	Modifikation		56 nmol	**GC Gehalt**	29 %
DNA-Typ	DNA		368 µg	**Tm (GC)**	39 °C
				ε (mM)	334,840

Zusammensetzung A= 4,0 C = 3,0 G = 3,0 T = 1,0 **Modi** = 10,0

Mod. 5'	**Mod. 5** 2'OMe-rU	**Mod. 7**
Mod. 3'	**Mod. 6** 2'OMe-rA	**Mod. 8** 2'OMe-rG

4	94B2						
5'-7g5 u6g 7a8 a6a 7a6 a6g 8a8-3'							
IDO	16911	**OD**	32,7 **bei**	260 nm	**Länge**	21-mer	
Scale	0,2 µmol	**Menge**		96665 pmol	**Molekular Gew.**	6555,12	
Reinigung	Modifikation			97 nmol	**GC Gehalt**	14 %	
DNA-Typ	DNA			634 µg	**Tm (GC)**	34 °C	
					ε (mM)	338,280	
Zusammensetzung	A=6,0	C=0,0	G=3,0	T=1,0	**Modi** = 11,0		
Mod. 5' **Mod. 3'**		**Mod. 5** 2'OMe-rU **Mod. 6** 2'OMe-rA			**Mod. 7** 2'OMe-rC **Mod. 8** 2'OMe-rG		

EXHIBIT 2 of
EXHIBIT A

[redacted] ▸ 96 well PTEN RNAi Transfection

Generic Cell Culture Assay For TaqMan Analysis

Assay: PTEN RNAi 's Date: [redacted]

Day 1
- Set 2 plates of HelaB cells in 96 well TC plates @ 2500 cells/well

Day 2

LIPID STOCK: NC 388 1 µg/ml
- Make 2.0 mls of 10X lipid stock solution in Complexing Media

LIPID:

1X	10X	ul lipid	ul complex media
1.0	10	60	1980

COMPLEX: RNAi stock 2.5nM
- Prepare 10X GB complex in 96 well polystyrene U-bottom plate 40ul each

GB:

1X	10X	ul stock	ul complex media
40	400	16	84

- Add 40ul of 10X lipid to the wells
- Shake plate
- Complex at 37c CO_2 incubator for 15-30 minutes
- Aspirate culture media from cells; add 80ul of new media (no antibiotics!)
- Add 10ul per well of the complex in each triplicate on 96 well plate
- Incubate in CO_2 incubator at 37° for 24 hours

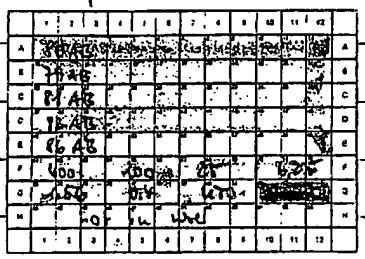
Day 3
- Aspirate media completely from wells, no wash. Lyse each well in 100ul RLT lysis buffer from Qiagen kit.

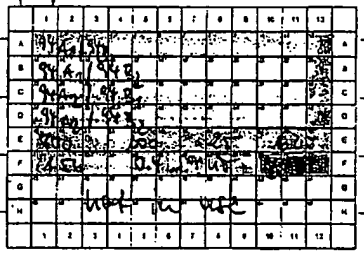
A	80 AB										
B	78 AB										
C	81 AB										
D	82 AB										
E	86 AB										
F											
G											
H											

40nM / 10nM / 2.5nM

A	94A1/94B1										
B	94A1/94B2										
C	94A2/94B1										
D	94A2/94B2										
E											
F											
G											
H											

[redacted] ▸ TaqMan 2x PTEN / pMoc

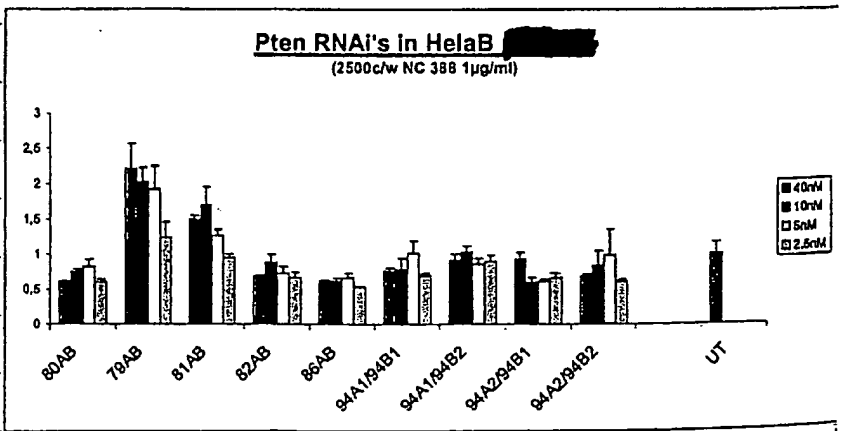




! Wiederholung d. Transfection erforderlich, da beim RNA Präp was schief gelaufen ist

2,5nM

73	0,09806335	0,60995611	0,03763394
83	0,32695876	1,24788547	0,21298051
87	0,08411727	0,95747478	0,04783074
88	0,09528035	0,67125114	0,07974706
81	0,05932739	0,53462942	0,01397018
87	0,17560172	0,69505888	0,04158917
83	0,0818074	0,89987118	0,09701874
81	0,03013899	0,86374898	0,06992218
83	0,36815269	0,81253536	0,0346389
89	0,11221274		
83	0,23545446		



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Read and Understood By

M. Techtmann	[redacted]	Melanie Baker	[redacted]
Signed	Date	Signed	Date

EXHIBIT B